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(54) Title: A METHOD FOR TREATING AUTOIMMUNE DISEASES USING ALPHA-INTERFERON AND/OR BETA-INTERFERON

(57) Abstract

A method is provided for preventing or treating an autoimmune disorder and/or recurrent autoimmune disorder in a transplant tissue in a mammal, which entails administering an effective amount of a single subtype of α- and/or β-interferon or a hybrid or analog of either or mixture thereof to the mammal.

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TITLE OF THE INVENTION

A METHOD FOR TREATING AUTOIMMUNE DISEASES USING ALPHA-INTERFERON AND/OR BETA-INTERFERON

BACKGROUND OF THE INVENTION

Field of the Invention:

The present invention relates to a method of preventing or treating autoimmune diseases using a single subtype of α-interferon, β-interferon or mixtures, including hybrids and/or analogs thereof.

Description of the Background:

The term "autoimmune disease" encompasses a wide variety of diseases. For example, the following diseases and conditions are examples of autoimmune diseases: Type 1 diabetes mellitus, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, Sjogren's syndrome, mixed connective tissue disease, ankylosis spondylitis, Reiter's syndrome, psoriatic arthritis, hypersensitivity vasculitis, ulcerative colitis, cirrhosis, autoimmune uveitis, myasthenia gravis, Buerger's disease, Kawasaki's disease, systemic necrotizing vasculitis, regional enteritis and hypoparathyroidism. At present, many of these diseases are neither preventable nor curable.

While studies have been made in an attempt to reverse the disease process for some of these diseases, beneficial results inhibiting these autoimmune diseases are usually only transient at best and are obtained with significant drug toxicity. For example, in attempting to treat or

reverse the disease process for patients having Type 1 diabetes mellitus with cyclosporin A, biopsy-proven nephrotoxic effects were observed in some patients after only one year of treatment. Unfortunately, more than one year of treatment appears to be necessary.

Moreover, recurrent autoimmune disease may occur in transplanted tissue and can be an important cause of transplant failure. For example, all patients with Type 1 diabetes mellitus receiving transplanted islet cells suffer from rejection thereof due, in part, to recurrent autoimmune disease.

Hence, a need exists for a method by which recurrent autoimmune disease could be prevented, and by which autoimmune diseases may be prevented and/or treated.

SUMMARY OF THE INVENTION

Accordingly, the present invention provides a method of preventing and/or treating autoimmune disorders by administering to a mammal, a single subtype of α-interferon, β-interferon or hybrids and/or analogs or mixtures thereof.

The present invention also provides a method of treating early asymptomatic stages of autoimmune disease in a mammal, which entails administering to a mammal, a single subtype of α-interferon, β-interferon or hybrids, analogs or mixtures thereof.

The above objects and oth r objects ar provided by a meth d of preventing or treating an autoimmune disorder in

a mammal or recurrent aut immune disease in transplant d tissues or cells, which entails administering to a mammal an effective amount of a single subtype of α-interferon, β-interferon or a mixture thereof, including hybrids and/or analogs or mixture thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 compares the development of diabetes mellitus in diabetes prone biobreeding (DP-BB) rats treated with α-IFN (400,000 units per dose) versus saline (control).

Figure 2 illustrates the effect of α-IFN (at 100,000 units/dose) treatment on the development of diabetes mellitus in DP-BB rats.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

For purposes of the present invention, the term "autoimmune disorder" means any disease or condition which is caused by or triggered by a breakdown of tolerance to autologous constituents, such as Type I diabetes mellitus, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, Sjogren's syndrome, mixed connective tissue disease, ankylosis spondylitis, Reiter's syndrome, psoriatic arthritis, hypersensitivity vasculitis, ulcerative colitis, cirrhosis, autoimmune uveitis, myasthenia gravis, Buerger's disease, Kawasaki's disease, systemic necrotizing vasculitis, regional enteritis and hypoparathyroidism.

In accordance with the present invention, it has been surprisingly discovered that single subtypes of α- and/or β-interferon or mixtures thereof, including hybrids and/or analogs or mixtures thereof, can be used with great advantage in preventing or treating autoimmune disorders.

It has also been discovered, in accordance with the present invention, that the same single subtypes of α- and/or β-interferon or mixtures thereof, including hybrids and/or analogs or mixtures thereof may be used to advantage in treating asymptomatic conditions which are present prior to the clinically apparent onset of autoimmune disease, or in treating recurrent autoimmune disease, such as Type I diabetes mellitus in transplanted pancreas or islet tissue.

In accordance with the present invention, the single α- and/or β-interferon subtype used may be a purified, naturally occurring or recombinant subtype, or it may be a hybrid of two or more subtypes or an analog thereof. Further, mixtures containing any two or more of the above may be used in accordance with the present invention.

In accordance with the present invention, many variations of the α-IFN and/or β-IFN subtypes, hybrids and/or analogs may be used. Furthermore, in accordance with the present invention, the α-IFN and/or β-IFN may originate from any mammalian species. Thus, for example, bovine β-IFN subtypes may be used in human therapy.

First, α-IFN and/or β-IFN subtypes may be used which have a length of 166 amino acid units, and which have at least 60% of the consensus sequence shown below in Tables 1

and 2, respectively. The remaining portion of the consensus sequence and any portion of or all of the non-consensus portions of any α-IFN or β-IFN may be substituted by any other amino acid, whether naturally occurring or not. By the term "non-consensus" portion or "non-consensus" amino acids is meant those amino acids which do not fall within the amino acids which are sequentially common to α-IFN and/or β-IFNs as shown in Table 1. Thus, for example, any α-IFN subtype from Table 1 and/or any β-IFN from Table 2 may be used as a starting model, and up to 40% of the consensus sequence may be substituted and up to 100% of the non-consensus sequence may be substituted by amino acids, such as, for example, glycine, alanine, valine, leucine, isoleucine, serine, threonine, cysteine, cystine, methionine, aspartic acid, glutamic acid, asparagine, glutamine, lysine, hydroxylysine, histidine, arginine, phenylalanine, tyrosine and tryptophan or even arnithine or citrulline.

Second, α-IFN and/or β-IFN subtypes, hybrids and/or analogs may be used which are less than 166 amino acid residues. In accordance with the present invention, the same rules will apply here as with the first variation above, except that the overall sequence length may be abbreviated to at least 70%, preferably at least 80% (132 or 133 units), and more preferably still to at least 90% (149 or 150 units).

Third, the α-IFN and/or β-IFN subtypes, hybrids and/or analogs or mixtures thereof of the present invention may be

incorporated as an "active portion" into a larger polypeptide or protein of the formula:

$$\epsilon-\gamma-\omega$$

wherein γ is the "active portion" as defined above, and ϵ and ω each independently represent from 0 to up to about 10,000 amino acids as defined above, with the proviso that the polypeptide or protein has the active portion, γ, topologically available at the surface of the polypeptide or protein in the event that it is folded in a three-dimensional structure. The design of such structures, such that a particular portion is available at the surface of the structure is within the skill of one in the art.

Further, in all of the above, the term "analog" means any active portion or sequence described herein having at least 60% of the same amino acids in the same sequence as any sequence described in Table 1 or Table 2 hereinbelow.

Generally, the term "interferon" refers to a family of proteins that confer non-specific resistance to a broad range of viral infections, affect cell proliferation and modulate immune responses. Three major interferons, α-, β- and γ have been identified based upon antigenic and physico-chemical properties, the nature of the inducer, and the cellular source from which they are derived. IFNs-α and -β, known collectively as Type I interferon, are structurally related, are stable at pH 2 and compete for the same cell surface receptor. IFN-γ, known as Type II

interferon, is structurally unrelated to Type I IFNs and is acid labile and has a different cell surface receptor.

α-IFN, refers to a family of highly homologous proteins that inhibit viral replication and cellular proliferation and which modulate immune responses. α-IFN is produced by many cells in the body, including peripheral blood leukocytes or lymphobloistoid cells upon exposure to live or inactivated virus, double-stranded RNA or bacterial products. Moreover, there are multiple subtypes of α-IFN which contain 165-166 amino acids and which have molecular weights of about 18,000 to 20,000 daltons.

β-IFN is a cytokine having antiviral, antiproliferative and immunomodulatory activities. Generally, β-IFN is a glycoprotein containing 166 amino acids having a molecular weight of about 20,000 daltons.

Generally, in accordance with the present invention, the amount of single subtype of α-IFN or β-IFN, hybrids, analogs or mixtures thereof administered per dose either prior to or after onset of disease is about $1X10^5$ units to about $75X10^6$ units with administrations being given from once per day to once per week. However, amounts may be used which are less than $1X10^5$ units, such as $5X10^4$ units or lower, or which are more than $75X10^6$ units, such as $10X10^7$ units or higher. Of course, the precise amount used will vary, depending upon the judgment of the attending physician, considering such factors as the age, w ight and condition of the patient. While any mammal may be treated,

such as dogs, cats, cows, horses or poultry, it is particularly desirable that the mammal treated be human.

Furthermore, in accordance with the present invention, the single subtype of α- and/or β-interferon or hybrids and/or analogs or mixtures thereof may be administered by any means of administration, such as orally, intravenously, intramuscularly, intraperitoneally or subcutaneously.

Generally, in accordance with the present invention any single subtype of α-IFN or β-IFN, hybrids and/or analogs or mixtures thereof, such as the human (HuIFN-α) subtypes may be used. The polypeptides or proteins may be used in either purified natural form or recombinant natural or hybrid or analog forms or mixtures thereof. While it is generally preferred to use species specific subtypes, non-species specific subtypes may also be used.

The amino acid sequences of many different α-IFN subtypes, such as Hu-IFNα are known. The following exemplary list is only illustrative, and by no means limitative.

Table 1

The Amino Acid Sequences of Different Hu IFN-α Subtypes Derived From cDNA or Genomic DNA Sequences*

	S1 S10	S20	S23	1 10	20
IFN-α consensus	MALSFSLLMA	VLVLSYKSIC	SLG	CDLPQTHSLG	NRRALILLAQ
IFN-α1	..SP.A...V	LV...C..S.	...	....E....D	...T.M....
IFN-αD	..SP.A...V	LV...C..S.	...	....E....D	...T.M....
IFN-α2	...T.A..V.	L....C..S.	.V.	....	S..T.M....
IFN-αA	...T.A..V.	L....C..S.	.V.	....	S..T.M....
IFN-αK(α6)	...P.A....	LV...C..S.	..D	....	H..TMM....
IFN-α5(G)	...P.V....	LV..NC....	...	.........S	...T.MIM..
IFN-αH1 (αH2)	...P...M..	LV...C..S.	...	.N.S.....N	...T.M.M..
IFN-αB2 (α8)	...T.Y..V.	LV......FS	...	....	....
IFN-αB	...T.Y.MV.	LV......FS	...	....	....
IFN-α4b	....	....	...	....	....
IFN-αC	....	....	...	....	........G.
IFN-αL (βα10)	....	.........*	...	.......T.R	........G.
IFN-αJ1 (α7)	..R......V	....	...	.........R	....
IFN-αJ2	..R......V	....	...	.........R	....
IFN-αf	....	....	...	....	....
IFN-αF	....	....	...	....	....
IFN-αWA	....	....	...	....	....
IFN-αGk-1	...P...M..	LV...C..S.	...	.N.S.....N	...T.MI...
IFN-α76	....	....	...	....	....

* The sequences, including the signal peptide, are presented in comparison with a consensus sequence, and residues are indicated only when they are different from the consensus sequence. In the latter, residues common to all listed sequences are underlined. Sequences with numeric designation are from Weissmann and collaborators, and sequences A to L are from Pestka, Goeddel et al. The Table utilizes standard one-letter amino acid symbols.

Table 1 (Cont'd)

	30	40	50	60	70
IFN-α consensus	MGRISPFSCL	KQRHDFGFPQ	EEFDGNQFQK	AQAISVLHEM	IQQTFNLFST
IFN-α1	.S....S...	M.....	.....	.P.....L	...I....T.
IFN-αD	.S....S...	M.....	.....	.P.....L	...I....T.
IFN-α2	.R...L....	.....	.....	.ET.P.....	...I.....
IFN-αA	.RK..L....	.....	.....	.ET.P.....	...I.....
IFN-αK(α6)	.R...L....	.....R...	.....	.E.....V	.....
IFN-α5(G)	.....	.....	.....	.....	.....
IFN-αH1 (αH2)	.R.....	.....E...	.....	.....	M.....
IFN-αB2 (α8)	.R.....	.....E...	....DK....	.....	.....
IFN-αB	.R.....	.....E...	....DK....	.....	.....
IFN-α4b	.....H....	.....E	.....H....	T.....	.....
IFN-αC	.....	.....RI..	.....	.....	.....
IFN-αL (βα10)	.....	.....RI..	.....	.....	.....
IFN-αJ1 (α7)	.....	....E.R..E	.....H....	T.....	.....
IFN-αJ2	.....	....E.R..E	.....H....	T.....	.....
IFN-αf	.....	...P...L..	.....	T.....	.....
IFN-αF	.....	.....	.....	.....	.....
IFN-αWA	.....H....	...Y.....	.V.....	.....AF...	.....
IFN-αGk-1	.....	.....	.....	.....	.....
IFN-α76	.....H....	.....E	.....H....	.....	.....

Table 1 (Cont'd)

	80	90	100	110
IFN-α consensus	KDSSAAWDES	LLEKFSTELY	QQLNDLEACV	JQEVGVEETP
IFN-α1	.....D	..D..C....	....	M..ER.G...
IFN-αD	.....D	..D..C....	....	M..ER.G...
IFN-α2	.....T	..D..Y....	....	..G...T...
IFN-αA	.....T	..D..Y....	....	..G...T...
IFN-αK(α6)	....V....R	..D.LY....	....	M...W.GG..
IFN-α5(G)	.....T...T	..D..Y....	.........M	M.....D..
IFN-αH1 (αH2)	.N.....T	....YI..F	.M......	....
IFN-αB2 (α8)	.....L..T	..DE.YI..D	.....S..	M.....I.S.
IFN-αB	.....L..T	..DE.YI..D	VLC	D.....I.S.
IFN-α4b	E.....EQ.	....	....	....
IFN-αC	E.....EQ.	....	....	....
IFN-αL (βα10)	E.....EQ.	.....I....	....	....
IFN-αJ1 (α7)	E.....EQ.	....	....	....
IFN-αJ2	E.....EQ.	....	.....N....	.....M....
IFN-αd	E.....EQ.	....	.....M....	....
IFN-αF	.....T.EQ.	....	....	T.....IA
IFN-αWA	.....T	..D..YI..F	....	M.....D..
IFN-αGχ-1	.....T...T	..D..Y....	.........M	....
IFN-α76	E.....EQ.	....	....	....

Table 1 (Cont'd)

	120	130	140	150	160	166
IFN-α consensus	LMNEDSILAV	RKYFQRITLY	LTEKKYSPCA	WEVVRAEIMR	SFSFSTNLQK	RLRRKD
IFN-α1	...A.....	K...R.....	....	....	.L.L.....E	.....E
IFN-αD	...V.....	K...R.....	....	....	.L.L.....E	.....E
IFN-α2	..K.....	....	.K.....	....	...L.....E	S..S.E
IFN-αA	..K.....	....	.K.....	....	...L.....E	S..S.E
IFN-αK(α6)	....	....	....	....	...S.R...E	.....E
IFN-α5(G)	...V....T.	....	....	....	...L.A...E	.....E
IFN-αH1 (αH2)	....	K.....	M.....	....	....	.....
IFN-αB2 (α8)	..Y.....	....	.....S..	....	...L.I....	..KS.E
IFN-αB	..Y.....	....	.....S..	....	...L.I....	..KS.E
IFN-α4b	...V.....	....	....	....	.L.....	.....
IFN-αC	....	....	.I.R.....	....	.L.....	.....
IFN-αL (βα10)	....	....	.I.R.....	....	.L.....	.....
IFN-αJ1 (α7)	.....F....	....	.M.....	....	.....K.	G.....
IFN-αJ2	.....F....	....	.M.....	....	....	.....
IFN-αd	....	....	....	....	.L.....	I.....
IFN-αF	...V.....	K.....	....	....	...L.KIF.E	.....E
IFN-αWA	....	....	.MG.....	....	....	G.....
IFN-αGχ-1	...V....T.	....	....	....	...L.A...E	.....E
IFN-α76	....	....	....	....	.L.....	.....

Table 2

Comparison of the Deduced Amino Acid Sequences, Including the Signal Peptides, of IFNβ of Human, Murine, and Bovine Origin.*

	S10	S20	S21
IFN-β consensus	MTχRCLLQχA	LLLCFSTTAL	S
Hu-IFN-β	..NK....I.	....	.
Mu-IFN-β	.NN.WI.HA.	F.........	.
Bo-IFN-β1	..Y.....MV	....	.
Bo-IFN-β2	..H.....MV	....	.
Bo-IFN-β3	..Y....PMV	....	.

	10	20	30
IFN-β consensus	χSYχLLχFQQ	RχSχχχCQKL	LχQLχχχχχχ
Hu-IFN-β	M..N..G.L.	.S.NFQ....	.W..NGRLEY
Mu-IFN-β	IN.KQ.QL.E	.TNIRK..E.	.E..NGKI..
Bo-IFN-β1	R..S..R...	.Q.LKE....	.G..PSTSQH
Bo-IFN-β2	R..S..R...	.R.LAL....	.R..PSTPQH
Bo-IFN-β3	R..S..R...	.R.AEV....	.G..HSTPQH

	40	50	60
IFN-β consensus	CLχχRMDFχχ	PEEMKQχQQF	QKEDAALχIY
Hu-IFN-β	..KD..N.DI	...I..L...	.......T..
Mu-IFN-β	N.TY.A..KI	....TE.KM.	..SYT.FA.Q
Bo-IFN-β1	..EA....QM	......E...	.....I.VM.
Bo-IFN-β2	..EA....QM	......A...	.....I.V..
Bo-IFN-β3	..EAK...QV	....N.A...	R....I.V..

	70	80	90
IFN-β consensus	EMLQNIFχIF	RχDFSSTGWN	ETIVEχLLχE
Hu-IFN-β	.......A..	.Q.S......	.....N..AN
Mu-IFN-β	.....V.LV.	.NN.......	....VR..D.
Bo-IFN-β1	.V..H..G.L	TR.......S	...I.D..K.
Bo-IFN-β2	....Q..N.L	TR.......S	...I.D..E.
Bo-IFN-β3	....Q..N.L	TR.......S	...I.D..V.

* The sequences are presented as they differ from a consensus sequence, and the amino acids of the consensus sequence that are common to all sequences are underlined. Positions where no clear consensus exists are indicated in the consensus sequence by "χ". The table is adapted from Pestka using the standard one-letter amino acid code.

Table 2 (Cont'd)

	100	110	120
IFN-β consensus	LYχQχNχLKT	VLEEKχEKEN	χTχGχχMSS--L
Hu-IFN-β	V.B.I.H...	.....L...D	F.R.KL...--.
Mu-IFN-β	.HQ.TVF...	.....Q.-.R	L.WE--...TA.
Bo-IFN-β1	..W.M.R.QP	IQK.IMQ.Q.	S.TEDTIV---P
Bo-IFN-β2	..E.M.H.EP	IQK.IMQ.Q.	S.M.DTTV---.
Bo-IFN-β3	..G.M.R.QP	IQK.IMQEQ.	F.M.DTTV---.

	130	140	150
IFN-β consensus	HLKχYYχRχχ	χYLKχKEYχχ	CAWTVVRVEI
Hu-IFN-β	...R..G.IL	H...A...SH	....I.....
Mu-IFN-β	...S..W.VQ	R...LMK.NS	Y..M...A..
Bo-IFN-β1	..GK..FNLM	Q..ES...DR	......Q.Q.
Bo-IFN-β2	..RK..FNLV	Q...S...NR	........Q.
Bo-IFN-β3	...K..FNLV	Q..ES...NR	........Q.

	160	166
IFN-β consensus	LRNFχFIχRL	TGYLRN
Hu-IFN-β	....Y..N..	.....
Mu-IFN-β	F...LI.R..	.RNFQ.
Bo-IFN-β1	.T.VS.LM..	...V.D
Bo-IFN-β2	....S.LT..	.....E
Bo-IFN-β3	.T..S.LM..	.AS..D

Table 1 provides a detailed sequence listing of various α-interferon subtypes, showing a consensus sequence for all. By "consensus sequence" is meant that sequence which is common to all α-IFN and β-IFN subtypes. See Tables 1 and 2. In accordance with the present invention, any α-interferon subtype may be used singly or in admixture with others or as hybrids and/or analogs or mixtures thereof as long as it contains, at the least, 60% of the consensus sequence shown in Table 1 as described above or a sequence which exhibits substantially the same α-IFN activity against autoimmune disease as a sequence having at least that portion of the consensus sequence.

Table 2 provides a comparison of detailed sequence listings for β-interferon of human, murine and bovine origin. In accordance with the present invention, any β-interferon subtype may be used as long as it contains at least 60% of the consensus sequence shown in Table 2 as described above or a sequence which exhibits substantially the same ß-IFN activity against autoimmune disease as a sequence having at least the consensus sequence.

In both Tables, the standard one-letter amino acid formulas are used. See Barker, Organic Chemistry of Biological Compounds, (Prentice Hall).

Generally, the phrase "substantially the same IFN activity" means an autoimmune process or disease inhibitory activity which may be anywhere from in excess of 1% to up to about 1000% of th same activity of a sequence having,

at least, about 60% of the consensus sequence of the sequences of Tables 1 or 2. Preferably, however, at least about 70%, and more preferably about 80% of the consensus sequence is present. It is most preferred, however, if at least about 90% of the consensus sequence, is present.

More preferably still, the other sequences are, in general, at least 95% or 100% homologous with those having, at least, the consensus sequence.

In the various subtypes of α-IFN and β-IFN, amino acid residues thereof may be substituted in the nonconsensus portion by other amine residues, such as, for example, glycine, alanine, valine, leucine, isoleucine,serine, threonine, cysteine, cystine, methionine, aspartic acid, glutamic acid, asparagine, glutamine, lysine, hydroxylysine, histidine, arginine, phenylalanine, tyrosine and tryptophan. However, these are only exemplary and other amino acids, such as ornithine or citrulline, for example, may also be used.

Further, hybrid interferons may be constructed and used, for example, from IFLrA and IFLrD interferon-coding sequences. If necessary, purification may be effected using a known monoclonal antibody to human leukocyte interferon. Such hybrid interferons are well known as described Pestka et al, Journal of Biological Chemistry, vol. 257, No. 19, Oct. 10, 1982, pp. 11497-11502, which article is incorporated herein in the entirety.

However, any hybrid α-IFN and/or β-IFN may be used. For example, other hybrids such as IFLrA1-62/D64-166 (Bgl II), IFLrA1-91/D93-166 (PUU II),

IFLrD1-92/A92-165 (PUU II),

IFLrD1-63/A63-165 (Bgl II), or

IFLrA1-62/D64-92/A92-165 (Bgl II-PUU II) may be used. These are only exemplary and others may be used.

Generally, analogs of the α-IFN and/or β-IFN or hybrid interferons or mixtures thereof described herein may also be used.

The present invention will now be further illustrated by reference to certain examples which are provided solely for purposes of illustration and are not intended to be limitative.

Studies were performed with diabetes prone-biobreeding (DP-BB) rats which constitute an acceptable model for Type 1 diabetes in humans.

EXAMPLE 1

This experiment was designed to determine if the administration of a hybrid α-interferon at a dose of 400,000 units can prevent the development of diabetes. See Figure 1.

DP-BB rats were divided into two groups; one being α-IFN treated (n = 7) and the other being saline treated (control) (n = 10).

rHu IFN-alpha-A/D Bgl II (Hoffmann La Roche) was administered at a dose of 400,000 units intraperitoneally three times a week beginning at approximately 40 days of age for about 8 weeks. Animals were diagnosed with diabetes when blood glucoses on two consecutive days exceeded 200 mg%. Animals were sacrificed at diagnosis of diabetes or at 120-130 days in the case of non-diabetic animals.

Using the survival curve analysis of Meier et al, the development of diabetes in the animals in the α-IFN-treated group was significantly lower than that for animals in the saline group ($p < 0.001$).

EXAMPLE 2

This experiment was designed to determine if the administration of a lower amount of the same α-interferon as used in Example 1 to DP-BB rats can alter the development of diabetes and insulitis.

Data from the treatment groups from two identically performed experiments are combined and described. (See Figure 2) DP-BB rats were divided into the following treatment groups: Group 1: normal saline (n=17); Group 2: α-IFN (35-40) day (n=15); and Group 3:α-IFN(28-30) day (n=6). Animals in the appropriate groups were administered (rHuIFN-alpha A/D Bgl II) 100,000 units intraperitoneally three times a week beginning at "35-40" days of age in Group II and "28-30" days of age in Group III. Treatment was discontinued after 6 weeks in the α-IFN(35-40) day

group and continued until sacrifice in the α-IFN(28-30) day group.

Using the survival curve analysis of Meier et al, the development of diabetes in the animals in the α-IFN-(35-40) day and A-IFN-(28-30) day groups were significantly slower than that for animals in the saline control group ($p < 0.001$). Thus, it is concluded that α-IFN administration at a dose of 100,000 units per injection prevents the development of diabetes in DP BB rats. It is noted that treatment was continued for six weeks in Group 2, but the effect thereof was long lasting and continued to the end of the experiment which was more than forty (40) days later.

Figure 2 also shows that doses of α-IFN lower than 400,000 units may be used to reduce the incidence of diabetes mellitus. For example, a dose of as low as about 100,000 units may be used effectively.

EFFECT OF A-IFN ADMINISTRATION ON PANCREATIC HISTOPATHOLOGY

Histopathologic examination of the pancreas revealed a decrease in the amount of mononuclear infiltration within the islet in animals treated with α-IFN than with saline. Thus, α-IFN administration appears to reduce the inflammatory response within the islet rather than inhibiting the islet destructive activity of immune cells within the isl ts.

As noted above, the present invention may be used to treat clinically apparent autoimmune disease, asymptomatic states which exist prior to clinically apparent autoimmune disease, and even "pre-states" or "pre-conditions" which exists in the mammalian body prior to the onset of the symptomatic states. These conditions may include risk factors for autoimmune disease.

As used herein, the term "risk factor" includes genetic markers, other physiological markers, such as those mentioned above, and also a combination thereof.

For example, the present invention may be used to treat the pre-diabetic state, which may be detected in humans by any one or all of the following, for example: i) the presence of serum islet cell antibodies, ii) the presence of serum insulin antibodies and iii) a depressed first phase insulin response (release) to intravenous glucose injection. Thus, the same treatment regime may be used for the preliminary conditions prior to disease as for the disease, itself.

Thus, in accordance with the present invention, various genetic markers may be used to identify mammals, particularly humans, which or who are at risk for one or more autoimmune diseases. Such genetic markers or tests for the detection of such genetic markers are well known to those skilled in the art. In essence, if a mammalian host or patient tests positive or exhibits a given level of risk for one or more of these markers or factors, then depending

upon the discretion of the treating physician or veterinarian, treatment may be commenced in accordance with the present invention.

Furthermore, the same treatment regimen as described above may be used in inhibiting recurrent autoimmune disease within transplanted tissue that contributes to graft failure. For example, α-IFN or β-IFN or the hybrids and/or analogs or mixtures thereof of the present invention may be used to advantage in inhibiting recurrent diabetes in the transplanted pancreas or islet cells in a patient having Type I diabetes. This is quite advantageous inasmuch as the conventional approach used in attempting to obtain such inhibition has entailed the administration of high doses of toxic drugs, such as cyclosporin A; steroids, such as prednisone; azathioprine, FK-506 and anti-leukocyte globulin, with only moderate success.

Thus, the present invention provides a method of treating asymptomatic conditions which precede onset of a clinically apparent autoimmune disease, which entails administering to a mammal presenting such symptoms and/or conditions an amount of a single subtype of α-interferon, β-interferon or a mixture, including hybrids, thereof effective to alleviate or reduce the symptoms and/or conditions.

Further, while each of the above methods may be practiced with any mammal, such as thos noted previously, these methods are particularly advantageous with humans.

The present invention also provides pharmaceutical compositions which includes at least one active ingredient and one or more pharmaceutically acceptable excipients. Generally, the term "active ingredient" is intended to mean any one or more subtypes, hybrids and/or analogs or mixtures thereof of the present invention, either alone or in combination with each other, and optionally with any other active ingredient which may be used to treat autoimmune diseases.

Thus, for example, any one of the α-IFN subtypes recited in Table 1 may be used alone or in combination with each other or in combination with the human β-IFN of Table 2 as an active ingredient. Additionally, any hybrids and/or analogs or mixtures thereof may be so used. Thus, for example, IFN-α1 may be mixed with IFN-α GK-1 in combination with an excipient and optionally with a conventional medicament for treating autoimmune disease.

The pharmaceutical composition may, for example, take the form of suspensions, solutions and emulsions of the active ingredient in aqueous or non-aqueous diluents, syrups, granulates or powders.

The diluents to be used in pharmaceutical compositions (e.g. granulates) adapted to be formed into tablets, dragees, capsules and pills include the following: (a) fillers and extenders, e.g. starch, sugars, mannitol, and silicic acid; (b) binding ag nts, .g. carboxymethyl cellulos and other cellulose derivatives, alginates,

gelatin and polyvinyl pyrrolidone; (c) moisturizing agents, e.g. glycerol; (d) disintegrating agents, e.g. agar-agar, calcium carbonate and sodium bicarbonate; (e) agents for retarding dissolution, e.g. paraffin; (f) resorption accelerators, e.g. quaternary ammonium compounds; (g) surface active agents, e.g. cetyl alcohol, glycerol monostearate; (h) adsorptive carriers, e.g. kaolin and bentonite; and (i) lubricants, e.g. talc, calcium and magnesium stearate and solid polyethyl glycols.

The tablets, dragees, capsules and pills formed from the pharmaceutical compositions of the invention can have the customary coatings, envelopes and protective matrices, which may contain pacifiers. They can be so constituted that they release the active ingredient only or preferably in a particular part of the intestinal tract, possibly over a period of time. The coatings, envelopes and protective matrices may be made, for example, of polymeric substances or waxes.

The ingredient can also be made up in microencapsulated form together with one or several of the above-mentioned diluents.

The diluents to be used in pharmaceutical compositions adapted to be formed into suppositories can, for example, be the usual water-soluble diluents, such as polyethylene glycols and fats (e.g. cocoa oil and high esters (e.g. C_{-14}-alcohol with C_{16}-fatty acid)) or mixtures of these diluents.

The pharmaceutical compositions which ar solutions and emulsions can, for example, contain the customary diluents, such as solvents, dissolving agents and emulsifiers; specific examples of such diluents are water, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (for example, ground nut oil), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitol or mixtures thereof.

For parenteral administration, solutions and emulsions should be sterile, and, if appropriate, blood-isotonic.

The pharmaceutical compositions which are suspensions can contain the usual diluents, such as liquid diluents, e.g. water, ethyl alcohol, propylene glycol, surface-active agents (e.g. ethoxylated isostearyl alcohols, polyoxyethylene sorbite and sorbitane esters), microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth or mixtures thereof.

All the pharmaceutical compositions according to the invention can also contain coloring agents and preservatives as well as perfumes and flavoring additions (e.g. peppermint oil and eucalyptus oil) and sweetening agents (e.g. saccharin).

The pharmaceutical compositions according to the invention generally contain fr m 0.5% to 90% of one or both the active ingredient by weight of the total composition.

In addition t a compound of the invention, the pharmaceutical compositions and medicaments according to the invention can also contain other pharmaceutically active compounds.

Any diluent in the medicaments of the present invention may be any of those mentioned above in relation to the pharmaceutical compositions of the present invention. Such medicaments may include well known pharmaceutically acceptable solvents generally having a molecular weight of less than about 200 as the single diluent.

The discrete coherent portions constituting the medicament according to the invention will generally be adapted by virtue of their shape or packaging for medical administration and may be, for example, any of the following: tablets (including lozenges and granulates), pills, dragees, capsules, suppositories and ampoules. Some of these forms may be made up for delayed release of the active ingredient. Some, such as capsules, include a protective envelope which renders the portions of the medicament physically discrete and coherent.

The production of the above-mentioned pharmaceutical compositions and medicaments may be carried out by any method known in the art, for example, by mixing the active ingredient(s) with the diluent(s) to form a pharmaceutical composition (e.g. a granulate) and then forming the composition into the medicament (e.g. tablets).

For pharmaceutical compositions intended for oral administration, the same may be coated using coating materials which are well known in the art. The amount of coating composition to be applied is generally such that not more than 4% of the drug must leach out into artificial saliva within a period of two minutes at 20-40°C. Among the most popular coating materials are: hydroxypropylcellulose, methylhydroxypropylcellulose, polyethylene oxide and polyvinyl pyrrolidone. These water-soluble polymers can be used alone or in admixture with water-insoluble polymers, such as ethylcellulose, polyvinylacetate, methylacrylate/methyl methacrylate, cellulose acetate phthalate, cellulose acetate butyrate, cellulose acetate propionate, polyvinylidene chloride, zein, and certain waxes as long as the resulting film is water-permeable. In the preferred embodiment, the coating material is applied to the pharmaceutical composition to the extent of at least 15% by weight of the complex. This insures almost complete taste masking. Where coating is done with water-soluble, film-formers, there is no substantial change of drug availability experienced in the gastro-intestinal juices between coated and uncoated drug/resin particles.

Generally, the various α-IFN and/or β-IFN subtypes, hybrids, or analogs described above may be either purchased commercially or may be produced in accordance with well known fermentative methods, such as are disclosed in

Current Protocols in Molecular Biology (Wiley 1987). Further, these subtypes, hybrids or analogs may be purchased from commercial entities, such as Roche Laboratories, Schering or Purdue Frederick, for example.

Moreover, the polypeptides of the present invention may be synthesized using a standard solid phase or liquid phase amino acid synthesis or may be synthesized in accordance with U.S. Patents 4,058,512 and 4,235,772 both of which are incorporated herein in the entirety. Also, these polypeptides may be readily obtained by custom synthesis from a variety of commercially available chemical supply companies.

Further, as indicated above, these polypeptides may be prepared by the fermentation of transformed microorganisms containing a synthetic gene coding for the same. Conventional techniques may be used for the synthesis of the appropriate gene and for the transformation of a host microorganism. As a host microorganism, E. coli, for example, may be used.

Finally, as noted above, the present polypeptides, as widely described above, may be used advantageously in treating hypoparathyroidism in mammals, particularly, in humans. In this aspect of the present invention, the same amounts used and modes of administration may be used as described above.

Having now described the present inv ntion it will be apparent to the artisan that many changes and modifications

may b made to th above-described embodiments without departing from the spirit and the scope of the present invention.

WHAT IS CLAIMED AND DESIRED TO BE SECURED BY UNITED STATES LETTERS PATENT

1. A method of preventing or treating an autoimmune disease in a mammal, which comprises administering to a mammal in need thereof an effective amount of at least one subtype of α- or β- interferon or a hybrid or analog of either or a mixture thereof.

2. The method of Claim 1, wherein said autoimmune disorder is Type 1 diabetes mellitus.

3. The method of Claim 1, wherein said mammal is a human.

4. The method of Claim 1, wherein said effective amount is about $1X10^5$ units to about $10X10^7$ units per administration.

5. The method of Claim 4, wherein the effective amount is about $1X10^6$ units to about $75X10^6$ units per administration.

6. The method of Claim 1, wherein a subtype of α-interferon or a mixture thereof is used.

7. The method of Claim 6, wherein said purified subtype of α-interferon or β-interferon is a purified naturally-occurring subtype thereof or a recombinant natural or recombinant hybrid subtype or analog thereof.

8. The method of Claim 7, wherein said subtype or subtypes have a sequence which exhibits an activity against autoimmun disease which is substantially similar to that exhibited by any one of the sequences of Tables 1 or 2.

9. Th method of Claim 8, wherein the sequenc or sequences of said subtype or subtypes is or are at least 60% homologous with a sequence containing the consensus sequence of Table 1 or 2.

10. The method of Claim 9, wherein the sequence or sequences of said subtype or subtypes is or are at least 80% homologous with a sequence containing the consensus sequence of Table 1 or 2.

11. The method of Claim 6, wherein said α-interferon is the recombinant rHuIFN alpha-A/D Bgl II.

12. A method of treating an asymptomatic preclinical autoimmune state in a mammal, which comprises administering to said mammal an effective amount of a single subtype of α- or β-interferon or a hybrid or analog of either or a mixture thereof.

13. The method of Claim 12, wherein said mammal is a human.

14. The method of Claim 12, wherein said pre-autoimmune condition is a pre-clinical state.

15. A method of inhibiting rejection of transplanted islet cells or a pancreas in a mammal having islet cells or a pancreas transplanted therein, which entails administering to the mammal an amount of a single subtype of α-interferon, β-interferon or a hybrid or analog thereof, or a mixture thereof effective to inhibit the rejection.

16. The method of Claim 15, wherein said mammal is a human.

17. The method of Claim 15, wherein a subtype of α-interferon or a mixture thereof is used.

18. The method of Claim 15, wherein said effective amount is about $5X10^4$ units to about $10X10^7$ units per administration.

19. The method of Claim 15, wherein said subtype or subtypes have a sequence which exhibits an activity against autoimmune disease which is substantially similar to that exhibited by a polypepetide containing the consensus sequence of Table 1 or Table 2.

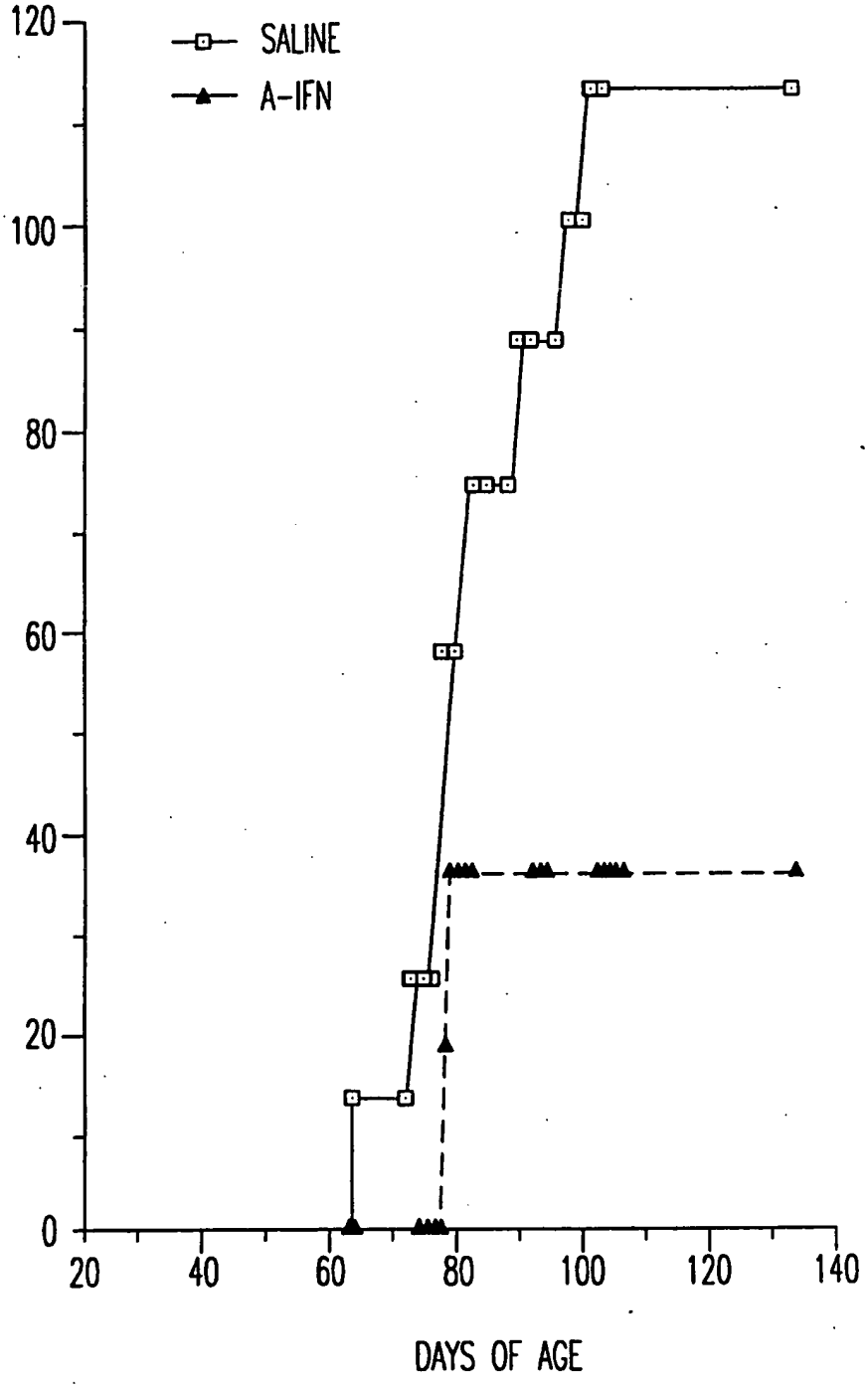
20. The method of Claim 19, wherein the sequence or sequences of subtype or subtypes is or are at least 60% homologous with a sequence containing the consensus sequence of Table 1 or Table 2.

21. The method of Claim 20, wherein the sequence or sequences of subtype or subtypes is or are at least 80% homologous with a sequence containing the consensus sequence of Table 1 or Table 2.

22. The method of Claim 15, wherein said α-interferon is the recombinant is rHuIFN alpha-A/D BglII.

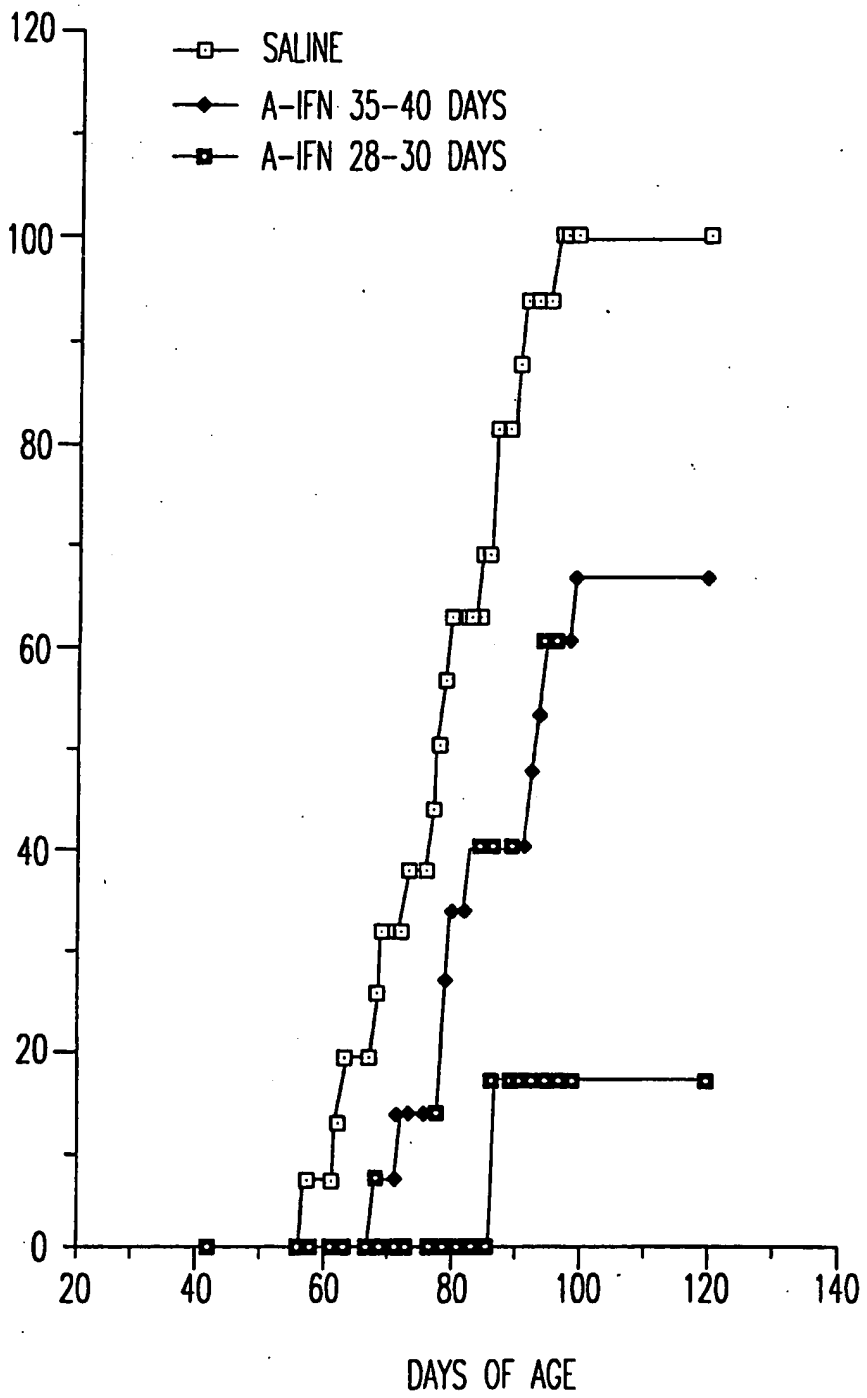
23. The method of Claim 15, wherein said rejection occurs as a consequence of recurrent diabetes.

FIG. 1


% OF RATS WITH DIABETES
SALINE
A-IFN
120
100
80
60
40
20
0
20
40
60
80
100
120
140
DAYS OF AGE

INCIDENCE OF DIABETES

FIG. 2



INTERNATIONAL SEARCH REPORT

In [redacted] application No.
PCT/US94/02154

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/00, 39/00; C07K 13/00; C12P 21/00
US CL : 424/85.6; 435/ 69.1; 514/2; 530/351
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85.6; 435/ 69.1; 514/2; 530/351

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Medline, Biosis, WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Archives of Neurology, Volume 43, Number 12, issued December 1986, Camenga et al, "Systemic Recombinant α-2 Interferon Therapy in Relapsing Multiple Sclerosis", pages 1239-1246, see abstract	1, 3-23
X	Rivista di Neurologia, Volume 59, Number 5, issued October 1989, Durelli et al, "Multiple Sclerosis. II. A Critical Assessment of Immunotherapy", pages 191-210, see abstract and page 199.	1, 3, 12
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Y		2, 4-11, 13-23
Y	Proceedings of National Academy of Science, USA, Volume 80, issued June 1983, Seghal et al, "Isolation of Novel Human Genomic DNA Clones Related to Human Interferon-β1 cDNA", pages 3632-3636, see abstract.	1-23

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
12 MAY 1994

Date of mailing of the international search report
JUN 03 1994

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/02154

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Quarterly Journal of Medicine, New Series 54, Number 214, issued February 1985, Tyrell, "Interferons and the Physician", pages 117-124, see pages 120-122.	1-23
Y	Journal of Biological Regulators and Homeostatic Agents, Volume 3, Number 2, issued 1989, Boucher et al, "Estimates of Normal Binding of a Human Recombinant Alpha Interferon to Peripheral Blood Mononuclear Cells from a Study Matching Healthy Subjects to Subjects with Insulin Dependent Diabetes", pages 47-49, see entire document.	1-23